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Date: February 6, 2002

By:

Carol A. SeePATENT
Docket No. GC634-2

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of)	
)	
Bron et al.)	Group Art Unit: 1653
)	
Serial No.: 09/954,737)	Examiner: Unassigned
)	
Filed: September 17, 2001)	
)	
For: Twin-Arginine Translocation in)	
Bacillus)	

Preliminary Amendment

Box Missing Parts
Commissioner for Patents
Washington, D.C. 20231

Sir:

Prior to examination, applicants respectfully request entry of the following amendments.

In the specification:

In the specification after page 54, insert pages 1-9 enclosed herewith.

On page 4, please replace the paragraph starting on line 28 with the following:

--Fig. 1A-B. **Tat components of *B. subtilis* and *E. coli*.** The amino acid sequences of Tat components of *B. subtilis* and *E. coli* as deduced from the SubtiList (<http://bioweb.pasteur.fr/Genolist/SubtiList.html>) and Colibri (<http://bioweb.pasteur.fr/Genolist/Colibri.html>) databases were used for comparisons. Identical amino acids [*], or conservative replacements [.] are marked. Putative transmembrane segments, indicated in gray shading, were predicted with the TopPred2 algorithm (34, 35) (A) Comparison of TatAc (YnzA), TatAd (YczB) and TatAy (YdiI) of *B. subtilis* (Bsu) with TatA, TatB and TatE of *E. coli* (Eco) (SEQ ID NO: 1-6). (B) Comparison of TatCd (YcbT) and TatCy (YdiJ) of *B. subtilis* with TatC of *E. coli* (SEQ ID NO: 7-9).--

On page 10, please replace the paragraph starting on line 5 with the following:

--FIG 14 Homologs of *B. clausii*. *B. subtilis* sequences (SEQ ID NO: 10 and 11, respectively) were used to BLAST search an in-house database of *B. clausii* genome.--

On page 17, please replace the paragraph starting on line 18 with the following:

--Tat Nucleic Acid and Amino Acid Sequences

The *TatCd* polynucleotide having the sequence corresponding to the amino acid sequence as shown in Figure 1B or 14 (SEQ ID NO: 9 and 11, respectively) encodes the *Bacillus subtilis* secretion factor TatCd. The *Bacillus subtilis* TatCd was identified via a FASTA search of *Bacillus subtilis* translated genomic sequences using a consensus sequence of TatC derived from *E.coli*. A FASTA search of *Bacillus subtilis* translated genomic sequences with the *E.coli* TatC sequence alone did not identify the *B. subtilis* TatCd. The present invention provides gram-positive *tatCd* polynucleotides which may be used alone or together with other secretion factors in a gram-positive host cell for the purpose of increasing the secretion of desired heterologous or homologous proteins or polypeptides.--

On page 19, please replace the paragraph starting on line 14 with the following:

--Also included within the scope of the present invention are novel gram-positive microorganism *tatC* polynucleotide sequences that are capable of hybridizing to part or all of the *tatC* nucleotide sequence of Figure 1B (SEQ ID NO: 7-9) or Figure 14 (SEQ ID NO: 11) under conditions of intermediate to maximal stringency. Hybridization conditions are based on the melting temperature (T_m) of the nucleic acid binding complex, as taught in Berger and Kimmel (1987, Guide to Molecular Cloning Techniques, Methods in Enzymology, Vol 152, Academic Press, San Diego CA) incorporated herein by reference, and confer a defined "stringency" as explained below.--

On page 20, please replace the paragraph starting on line 19 with the following:

--The *B. subtilis* *tatCd* polynucleotide corresponding to the amino acid sequence as shown in Figure 1B (SEQ ID NO: 9) or 14 (SEQ ID NO: 11) encodes *B. subtilis* TatCd. The present invention encompasses novel gram positive microorganism amino acid variants of the amino acid sequence shown in Figure 1B (SEQ ID NO: 9) or 14 (SEQ ID NO: 11) that are at least 80% identical, at least 90% identical and at least 95% identical to the sequence shown in

Figure 1 or 14 as long as the amino acid sequence variant is able to function by modulating secretion of proteins in gram-positive microorganisms.--

On page 20, please replace the paragraph starting on line 26 with the following:

--The secretion factor TatCd as shown in Figure 1B (SEQ ID NO: 9) was subjected to a FASTA (Lipmann Pearson routine) amino acid search against a consensus amino acid sequence for TatCd. The amino acid alignment is shown in Figure 1.--

On page 35, please replace the paragraph starting on line 9 with the following:

--Second, in contrast to *E. coli*, which contains a unique *tatC* gene (10), *B. subtilis* was shown to contain two paralogous *tatC*-like genes (*ie. ycbT* and *ydiJ*). The YcbT protein (245 residues), which was renamed TatCd, and the YdiJ protein (254 residues), which was renamed TatCy, showed significant similarity to the *E. coli* TatC protein (57% identical residues and conservative replacements in the three aligned sequences; Fig. 1B (SEQ ID NO: 7-9)). Like TatC of *E. coli*, TatCd and TatCy of *B. subtilis* have six potential transmembrane segments (Fig. 1B), and the amino-termini of these proteins are predicted to face the cytoplasm (data not shown).--

On page 37, please replace the paragraph starting on line 16 with the following:

--To construct *B. subtilis* *ltatCd*, the 5' region of the *tatCd* gene was amplified by PCR with the primers JJ14bT (5'-CCC AAG CTT ATG AAA GGG AGG GCT TTT TTG AAT GG-3' SEQ ID NO: 12) containing a *HindIII* site, and JJ15bT (5'-GCG GAT CCA AAG CTG AGC ACG ATC GG-3' SEQ ID NO: 13) containing a *BamHI* site. The amplified fragment was cleaved with *HindIII* and *BamHI*, and cloned in the corresponding sites of pMutin2 (Vagner et al. (1998) *Microbiol.* **144**, 3097-3104), resulting in pMICd1. *B. subtilis* *ltatCd* was obtained by a Campbell-type integration (single cross-over) of pMICd1 into the *tatCd* region of the chromosome.--

On page 37, please replace the paragraph starting on line 25 with the following:

--To construct *B. subtilis* *ltatCy*, the 5' region of the *tatCy* gene was amplified by PCR with the primers JJ03iJ (5'-CCC AAG CTT AAA AAG AAA GAA GAT CAG TAA GTT AGG ATG-3' SEQ ID NO: 14) containing a *HindIII* site, and JJ04iJ (5'-GCG GAT CCA AGT CCT GAG AAA TCC G-3' SEQ ID NO: 15) containing a *BamHI* site. The amplified fragment was cleaved with *HindIII* and *BamHI*, and cloned in the corresponding sites of pMutin2, resulting in pMICy1. B.

subtilis *ItatCy* was obtained by a Campbell-type integration (single cross-over) of pMICy1 into the *tatCy* region of the chromosome.--

On page 38, please replace the paragraph starting on line 4 with the following:

--To construct *B. subtilis* Δ *tatCd*, the *tatCd* gene was amplified by PCR with primer JJ33Cdd (5'-GGA ATT CGT GGG ACG GCT ACC-3' SEQ ID NO: 16) containing an *EcoRI* site and 5' sequences of *tatCd*, and primer JJ34Cdd (5'-CGG GAT CCA TCA TGG GAA GCG-3' SEQ ID NO: 17) containing a *BamHI* site and 3' sequences of *tatCd*. Next, the PCR-amplified fragment was cleaved with *EcoRI* and *BamHI* and ligated into the corresponding sites of pUC21, resulting in pJCd1. Plasmid pJCd2 was obtained by replacing an internal *BclI*-*AccI* fragment of the *tatCd* gene in pJCd1 with a pDG792-derived Km resistance marker, flanked by *BamHI* and *Clal* restriction sites. Finally, *B. subtilis* Δ *tatCd* was obtained by a double cross-over recombination event between the disrupted *tatCd* gene of pJCd2 and the chromosomal *tatCd* gene.--

On page 38, please replace the paragraph starting on line 15 with the following:

--To construct *B. subtilis* Δ *tatCy*, the *tatCy* gene was amplified by PCR with primer JJ29Cyd (5'-GGG GTA CCG GAA AAC GCT TGA TCA GG-3' SEQ ID NO: 18) containing a *KpnI* site and 5' sequences of *tatCy*, and primer JJ30Cyd (5'-CGG GAT CCT TTG GGC GAT AGC C-3' SEQ ID NO: 19) containing a *BamHI* site and 3' sequences of *tatCy*. Next, the PCR-amplified fragment was cleaved with *KpnI* and *BamHI* and ligated into the *Asp718* and *BamHI* sites of pUC21, resulting in pJCy1. Plasmid pJCy2 was obtained by ligating a pDG1726-derived Sp resistance marker, flanked by *PstI* restriction sites, into the unique *PstI* site of the *tatCy* gene in pJCy1. Finally, *B. subtilis* Δ *tatCy* was obtained by a double cross-over recombination event between the disrupted *tatCy* gene of pJCy2 and the chromosomal *tatCy* gene.--

On page 44, please replace the paragraph starting on line 1 with the following:

--To construct pAR3*phoD*, the *phoD* gene including its ribosome binding site was amplified from the chromosome of *B. subtilis* strain 168 by PCR using the primers P1 (5'- GAG GAT CCA TGA GGA GAG AGG GGA TCT TGA ATG GCA TAC GAC-3' SEQ ID NO: 20) containing a *BamHI* site, and P2 (5'-CGA TCC TGC AGG ACC TCA TCG GAT TGC-3' SEQ ID NO: 21) containing a *PstI* site. The amplified fragment was cleaved with *BamHI* and *PstI*, and

cloned in the corresponding sites of pAR3. The resulting plasmid pAR3*phoD* allowed the arabinose inducible expression of wild type *phoD* in *E. coli*.--

On page 44, please replace the paragraph starting on line 9 with the following:

--To construct a gene fusion between *bla* and *phoD* genes, the signal sequence less *phoD* was amplified using primers P3 (5'-GTA GGA TCC GCG CCT AAC TTC TCA AGC-3' SEQ ID NO: 22) containing a *Bam*HI site and primer P2 containing a *Pst*I site. The amplified fragment was cleaved with *Bam*HI and *Pst*I, and cloned in the corresponding sites of pUC19, resulting in plasmid pUC19'*phoD*. Next, the 5' region of TEM- β -lactamase encoding its signal sequence was amplified from plasmid pBR322 by PCR with primers B1 (5'-ATA GAA TTC AAA AAG GAA GAG TAT G-3' SEQ ID NO: 23) containing an *Eco*RI site, and primer B2 (5'-CTG GGG ATC CAA AAA CAG GAA GGC-3' SEQ ID NO: 24) containing a *Bam*HI site. The amplified PCR fragment was cleaved with *Bam*HI and *Eco*RI and inserted into pUC19'*phoD*, cleaved with the same restriction enzymes, resulting in plasmid pUC19*bla-phoD*. For easy selection of recombinant clones plasmid pOR124, containing a tetracycline resistance gene was inserted 3' of the *bla-phoD* gene fusion using an unique *Pst*I site. From the resulting plasmid pUC19*bla-phoD*-Tc an *Eco*RI-*Bgl*II fragment containing *bla-phoD* and the tetracycline resistance gene of pOR124 was isolated and inserted into pMUTIN2 cleaved with *Eco*RI and *Bam*HI. At plasmid pMutin2*bla-phoD* the *bla-phoD* gene fusion is under control of the IPTG-inducible P_{SPAC} promoter.--

On page 44, please replace the paragraph starting on line 27 with the following:

--To construct a gene fusion consisting of the signal sequence of *phoD* and *lacZ*, a DNA fragment encoding the signal peptide of PhoD and the translational start site of *phoD* was amplified by PCR with primer P1 containing a *Bam*HI site and primer P4 (5'-GAG AAG GTC GAC GCA GCA TTT ACT TCA AAG GCC CC-3' SEQ ID NO: 25) containing a *Sal*I site, and inserted into the corresponding sites of pOR124 resulting in plasmid pOR124*phoD*'. Next the *lacZ* gene lacking nine 5' terminal codons was amplified using primers L1 (5'-ACC GGG TCG ACC GTC GTT TTA CAA CG-3' SEQ ID NO: 26) containing a *Sal*I site and primer L2 (5'-GGG AAT TCA TGG CCT GCC CGG TT-3' SEQ ID NO: 27) containing an *Eco*RI site and subsequently inserted into the corresponding sites of pOR124*phoD*. The resulting plasmid pOR124*phoD-lacZ* was linearized with *Bam*HI and inserted into pAR3 cleaved with *Bgl*II. The resulting plasmid pAR3*phoD-lacZ* allows the arabinose inducible expression of the *phoD-lacZ* gene fusion.--

On page 45, please replace the paragraph starting on line 11 with the following:

--To obtain a plasmid mediating an inducible overexpression of *tatA_d tatC_d* of *B. subtilis*, the DNA region containing these genes including their ribosome binding sites was amplified by PCR with the primers T1 (5'-CAA GGA TCC CGA ATT AAG GAG TGG-3' SEQ ID NO: 28) containing a *Bam*HI site and primer T2 (5'-GGT CTG CAG CTG CAC TAA GCG GCC GCC-3' SEQ ID NO: 29) containing a *Pst*I site. The amplified fragment was cleaved with *Bam*HI and *Pst*I and cloned into the corresponding sites of pQE9 (QIAGEN), resulting in pQE9*tatA_d/C_d*--

GC634-2 seqpa

REMARKS

Entry of the above amendment prior to examination is respectfully requested.

Attached hereto is a marked-up version of the changes made to the specification and claims. The attached pages are captioned "**Version with Markings to Show Changes Made.**"

The specification has been amended in accordance with 37 C.F.R. §1.821 through 1.825 to add the Sequence Listing.

The specification has been amended in accordance with C.F.R. §1.821(d) to add SEQ ID NO:s.

If in the opinion of the Examiner a telephone conference would expedite the prosecution of the subject application, the Examiner is encouraged to call the undersigned at (650) 846-7500.

Respectfully submitted,

Date: Feb 6, 2002

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Version with Markings to Show Changes Made

Fig. 1A-B. **Tat components of *B. subtilis* and *E. coli*.** The amino acid sequences of Tat components of *B. subtilis* and *E. coli* as deduced from the SubtiList (<http://bioweb.pasteur.fr/Genolist/SubtiList.html>) and Colibri (<http://bioweb.pasteur.fr/Genolist/Colibri.html>) databases were used for comparisons. Identical amino acids [*], or conservative replacements [.] are marked. Putative transmembrane segments, indicated in gray shading, were predicted with the TopPred2 algorithm (34, 35) (A) Comparison of TatAc (YnzA), TatAd (YczB) and TatAy (YdiI) of *B. subtilis* (Bsu) with TatA, TatB and TatE of *E. coli* (Eco) (SEQ ID NO: 1-6). (B) Comparison of TatCd (YcbT) and TatCy (YdiJ) of *B. subtilis* with TatC of *E. coli* (SEQ ID NO: 7-9).

FIG 14 Homologs of *B. clausii*. *B. subtilis* sequences (SEQ ID NO: 10 and 11, respectively) were used to BLAST search an in-house database of *B. clausii* genome.

Tat Nucleic Acid and Amino Acid Sequences

The *TatCd* polynucleotide having the sequence corresponding to the amino acid sequence as shown in Figure 1B or 14 (SEQ ID NO: 9 and 11, respectively) encodes the *Bacillus subtilis* secretion factor TatCd. The *Bacillus subtilis* TatCd was identified via a FASTA search of *Bacillus subtilis* translated genomic sequences using a consensus sequence of TatC derived from *E. coli*. A FASTA search of *Bacillus subtilis* translated genomic sequences with the *E. coli* TatC sequence alone did not identify the *B. subtilis* TatCd. The present invention provides gram-positive *tatCd* polynucleotides which may be used alone or together with other secretion factors in a gram-positive host cell for the purpose of increasing the secretion of desired heterologous or homologous proteins or polypeptides.

Also included within the scope of the present invention are novel gram-positive microorganism *tatC* polynucleotide sequences that are capable of hybridizing to part or all of the *tatC* nucleotide sequence of Figures 21B (SEQ ID NO: 7-9) or Figure 14 (SEQ ID NO: 11) under conditions of intermediate to maximal stringency. Hybridization conditions are based on the melting temperature (T_m) of the nucleic acid binding complex, as taught in Berger and Kimmel (1987, Guide to Molecular Cloning Techniques, Methods in Enzymology, Vol 152, Academic Press, San Diego CA) incorporated herein by reference, and confer a defined "stringency" as explained below.

The *B. subtilis* *tatCd* polynucleotide corresponding to the amino acid sequence as shown in Figure 1B (SEQ ID NO: 9) or 14 (SEQ ID NO: 11) encodes *B. subtilis* TatCd. The present

invention encompasses novel gram positive microorganism amino acid variants of the amino acid sequence shown in Figure 1B (SEQ ID NO: 9) or 14 (SEQ ID NO: 11) that are at least 80% identical, at least 90% identical and at least 95% identical to the sequence shown in Figure 1 or 14 as long as the amino acid sequence variant is able to function by modulating secretion of proteins in gram-positive microorganisms.

The secretion factor TatCd as shown in Figure 1B (SEQ ID NO: 9) was subjected to a FASTA (Lipmann Pearson routine) amino acid search against a consensus amino acid sequence for TatCd. The amino acid alignment is shown in Figure 1.

Second, in contrast to *E. coli*, which contains a unique *tatC* gene (10), *B. subtilis* was shown to contain two paralogous *tatC*-like genes (ie. *ycbT* and *ydiJ*). The YcbT protein (245 residues), which was renamed TatCd, and the YdiJ protein (254 residues), which was renamed TatCy, showed significant similarity to the *E. coli* TatC protein (57% identical residues and conservative replacements in the three aligned sequences; Fig. 1B (SEQ ID NO: 7-9)). Like TatC of *E. coli*, TatCd and TatCy of *B. subtilis* have six potential transmembrane segments (Fig. 1B), and the amino-termini of these proteins are predicted to face the cytoplasm (data not shown).

To construct *B. subtilis* *ltatCd*, the 5' region of the *tatCd* gene was amplified by PCR with the primers JJ14bT (5'-CCC AAG CTT ATG AAA GGG AGG GCT TTT TTG AAT GG-3' SEQ ID NO: 12) containing a *HindIII* site, and JJ15bT (5'-GCG GAT CCA AAG CTG AGC ACG ATC GG-3' SEQ ID NO: 13) containing a *BamHI* site. The amplified fragment was cleaved with *HindIII* and *BamHI*, and cloned in the corresponding sites of pMutin2 (Vagner et al. (1998) *Microbiol.* **144**, 3097-3104), resulting in pMICd1. *B. subtilis* *ltatCd* was obtained by a Campbell-type integration (single cross-over) of pMICd1 into the *tatCd* region of the chromosome.

To construct *B. subtilis* *ltatCy*, the 5' region of the *tatCy* gene was amplified by PCR with the primers JJ03iJ (5'-CCC AAG CTT AAA AAG AAA GAA GAT CAG TAA GTT AGG ATG-3' SEQ ID NO: 14) containing a *HindIII* site, and JJ04iJ (5'-GCG GAT CCA AGT CCT GAG AAA TCC G-3' SEQ ID NO: 15) containing a *BamHI* site. The amplified fragment was cleaved with *HindIII* and *BamHI*, and cloned in the corresponding sites of pMutin2, resulting in pMICy1. *B. subtilis* *ltatCy* was obtained by a Campbell-type integration (single cross-over) of pMICy1 into the *tatCy* region of the chromosome.

To construct *B. subtilis* Δ *tatCd*, the *tatCd* gene was amplified by PCR with primer JJ33Cdd (5'-GGA ATT CGT GGG ACG GCT ACC-3' [SEQ ID NO: 16](#)) containing an *EcoRI* site and 5' sequences of *tatCd*, and primer JJ34Cdd (5'-CGG GAT CCA TCA TGG GAA GCG-3' [SEQ ID NO: 17](#)) containing a *Bam*HI site and 3' sequences of *tatCd*. Next, the PCR-amplified fragment was cleaved with *EcoRI* and *Bam*HI and ligated into the corresponding sites of pUC21, resulting in pJCd1. Plasmid pJCd2 was obtained by replacing an internal *Bcl*I-*Acc*I fragment of the *tatCd* gene in pJCd1 with a pDG792-derived Km resistance marker, flanked by *Bam*HI and *Cl*aI restriction sites. Finally, *B. subtilis* Δ *tatCd* was obtained by a double cross-over recombination event between the disrupted *tatCd* gene of pJCd2 and the chromosomal *tatCd* gene.

To construct *B. subtilis* Δ *tatCy*, the *tatCy* gene was amplified by PCR with primer JJ29Cyd (5'-GGG GTA CCG GAA AAC GCT TGA TCA GG-3' [SEQ ID NO: 18](#)) containing a *Kpn*I site and 5' sequences of *tatCy*, and primer JJ30Cyd (5'-CGG GAT CCT TTG GGC GAT AGC C-3' [SEQ ID NO: 19](#)) containing a *Bam*HI site and 3' sequences of *tatCy*. Next, the PCR-amplified fragment was cleaved with *Kpn*I and *Bam*HI and ligated into the *Asp*718 and *Bam*HI sites of pUC21, resulting in pJCy1. Plasmid pJCy2 was obtained by ligating a pDG1726-derived *Sp* resistance marker, flanked by *Pst*I restriction sites, into the unique *Pst*I site of the *tatCy* gene in pJCy1. Finally, *B. subtilis* Δ *tatCy* was obtained by a double cross-over recombination event between the disrupted *tatCy* gene of pJCy2 and the chromosomal *tatCy* gene.

To construct pAR3*phoD*, the *phoD* gene including its ribosome binding site was amplified from the chromosome of *B. subtilis* strain 168 by PCR using the primers P1 (5'- GAG GAT CCA TGA GGA GAG AGG GGA TCT TGA ATG GCA TAC GAC-3' [SEQ ID NO: 20](#)) containing a *Bam*HI site, and P2 (5'-CGA TCC TGC AGG ACC TCA TCG GAT TGC-3' [SEQ ID NO: 21](#)) containing a *Pst*I site. The amplified fragment was cleaved with *Bam*HI and *Pst*I, and cloned in the corresponding sites of pAR3. The resulting plasmid pAR3*phoD* allowed the arabinose inducible expression of wild type *phoD* in *E. coli*.

To construct a gene fusion between *bla* and *phoD* genes, the signal sequence less *phoD* was amplified using primers P3 (5'-GTA GGA TCC GCG CCT AAC TTC TCA AGC-3' [SEQ ID NO: 22](#)) containing a *Bam*HI site and primer P2 containing a *Pst*I site. The amplified fragment was cleaved with *Bam*HI and *Pst*I, and cloned in the corresponding sites of pUC19, resulting in plasmid pUC19'*phoD*. Next, the 5' region of TEM- β -lactamase encoding its signal sequence

was amplified from plasmid pBR322 by PCR with primers B1 (5'-ATA GAA TTC AAA AAG GAA GAG TAT G-3' SEQ ID NO: 23) containing an *EcoRI* site, and primer B2 (5'-CTG GGG ATC CAA AAA CAG GAA GGC-3' SEQ ID NO: 24) containing a *BamHI* site. The amplified PCR fragment was cleaved with *BamHI* and *EcoRI* and inserted into pUC19'*phoD*, cleaved with the same restriction enzymes, resulting in plasmid pUC19*bla-phoD*. For easy selection of recombinant clones plasmid pOR124, containing a tetracycline resistance gene was inserted 3' of the *bla-phoD* gene fusion using an unique *PstI* site. From the resulting plasmid pUC19*bla-phoD*-Tc an *EcoRI*-*BglII* fragment containing *bla-phoD* and the tetracycline resistance gene of pOR124 was isolated and inserted into pMUTIN2 cleaved with *EcoRI* and *BamHI*. At plasmid pMutin2*bla-phoD* the *bla-phoD* gene fusion is under control of the IPTG-inducible P_{SPAC} promoter.

To construct a gene fusion consisting of the signal sequence of *phoD* and *lacZ*, a DNA fragment encoding the signal peptide of *PhoD* and the translational start site of *phoD* was amplified by PCR with primer P1 containing a *BamHI* site and primer P4 (5'-GAG AAG GTC GAC GCA GCA TTT ACT TCA AAG GCC CC-3' SEQ ID NO: 25) containing a *Sall* site, and inserted into the corresponding sites of pOR124 resulting in plasmid pOR124*phoD'*. Next the *lacZ* gene lacking nine 5' terminal codons was amplified using primers L1 (5'-ACC GGG TCG ACC GTC GTT TTA CAA CG-3' SEQ ID NO: 26) containing a *Sall* site and primer L2 (5'-GGG AAT TCA TGG CCT GCC CGG TT-3' SEQ ID NO: 27) containing an *EcoRI* site and subsequently inserted into the corresponding sites of pOR124*phoD*. The resulting plasmid pOR124*phoD-lacZ* was linearized with *BamHI* and inserted into pAR3 cleaved with *BglII*. The resulting plasmid pAR3*phoD-lacZ* allows the arabinose inducible expression of the *phoD-lacZ* gene fusion.

To obtain a plasmid mediating an inducible overexpression of *tatA_d* *tatC_d* of *B. subtilis*, the DNA region containing these genes including their ribosome binding sites was amplified by PCR with the primers T1 (5'-CAA GGA TCC CGA ATT AAG GAG TGG-3' SEQ ID NO: 28) containing a *BamHI* site and primer T2 (5'-GGT CTG CAG CTG CAC TAA GCG GCC GCC-3' SEQ ID NO: 29) containing a *PstI* site. The amplified fragment was cleaved with *BamHI* and *PstI* and cloned into the corresponding sites of pQE9 (QIAGEN), resulting in pQE9*tatA_d/C_d*.